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Structure and dynamics of populations of *Rhabditis* (Nematoda: Rhabditidae) from forest soil

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With 3 figures

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Contents

1. Introduction

Nematode species belonging to the family Rhabditidae Oerley 1880, have been much studied in laboratory cultures (Dougherty 1960, Kämpfe and Wagner 1964, Sohlenius 1969, Yeates 1970). However ecological applications of these results to field conditions are few and quantative aspects of population ecology in field have not been much considered.

Outstanding characteristics of many species of Rhabditidae are their high growth rate and high rate of reproduction (Sohlenius 1968, 1969). Many, if not all, members of the family are able to form a resistant juvenile form commonly referred to as dauer larva. These are formed when the conditions become unfavourable and are considered to be means of dispersal and survival during adverse conditions (Osche 1954, 1963). The dauer larvae do not ingest any food and can survive for long periods in the absence of suitable food supply.

Earlier studies on agar reared populations of rhabditid nematodes have shown that the frequency of dauer larvae increases as the cultures get old and the growth conditions are impaired (Sohlenius 1969). Thus the relative frequency of dauer larvae within a population may indicate if the environmental conditions for population growth are favourable or not. In the present work it is shown that the proportion of active stages and dauer larvae changes during natural and experimental conditions in a species of *Rhabditis* Dujardin, 1845 (Osche, 1952). One purpose was to see if these proportions could be used to indicate the growth conditions for field populations of this species.

The work is closely connected with an earlier study on *Aerobeloides nanus* (de Man 1880) [Sohlenius 1973].

2. Material and methods

Rhabditis populations were sampled in five localities in central Sweden. In Uppland samples were taken from a mixed pine forest (Sollentuna locality 1), an aspen forest (Sollentuna locality 2) and an arable field with wheat (Danderyd locality 3) and in Småland from a forest of Scotch fir (*Pinus silvestris* L.) (Linderås locality 4) and a forest of common fir [*Picea abies* (L.) Karst.; Adelöv locality 5].

The most extensive sampling was done in locality 1 (mixed pine forest Sollentuna). This locality and the variations in temperature are described in an earlier paper (Sohlenius 1973). The animals were sampled from the raw humus layer in a monthly sampling program and extracted with a modified Baermann method as described earlier (Sohlenius 1973).

In the same locality (no. 1) the effect of the enrichment with organic material upon the *Rhabditis* population was studied. Five bags of nylon-netting with boiled fungal fruiting bodies (from study site) and 2 with feces (from Homo) were buried in the soil. At different times after application to soil samples were taken from the adjacent soil and from the interior of the bags. More detailed information regarding this experiment, which was also used for studies on *Acrobeloides nanus* is found in Sohlenius 1973.

Agar cultures (soil extract agar and Nigon's agar) were started according to methods described earlier (Sohlenius 1968).

3. Identity of species

When nematode suspensions from locality 1 (mixed pine forest Sollentuna) were transferred to soil agar plates there was quite often a rapid population growth of nematodes belonging to *Rhabditis*. Several such inoculations were done at different seasons and when *Rhabditis* populations grew out they always belonged to a species that reproduced uniparentally. No morphological differences were found between animals from agar reared populations and animals from field populations. Therefore it is probable that just one species was dominating in the locality. On some occasions males were found and these show that the species belongs to what Osche (1952) calls the *maupasi* group. According to Dougherty (1955) this group belongs to the subgenus *Rhabditis* Dujardin, 1845 (Osche, 1952). The species of the *maupasi* group are hard to identify but obviously the species from locality 1 resembles very much *Rhabditis maupasi* Seurat in Maupas 1919 and *Rhabditis terricola* Dujardin 1845 (syn. *R. aspera* Bütschli 1873). The only pronounced difference was the rareness of males. According to Osche (1952) the maupasi group contains many hermaphroditic species.

Also from locality 2 (aspen forest Sollentuna) populations of species belonging to the maupasi group were isolated. Populations of nematode species belonging to this group have been isolated from other soils in Uppland. I have not found any clear differences in morphology and dimensions between animals from these different populations, so it is quite possible that there is just one species which has a quite wide distribution.

4. Results and discussion

4.1. The field population

In the forest localities *Rhabditis* spp. constituted a very small proportion of the nematode fauna and had a rather patchy distribution. In the arable soil (locality 3) it constituted an important proportion of the nematode fauna (Table 1).

Table 1 Abundance and frequency of *Rhabditis* spec. in soil from some different localities

	Locality				
	Uppland			Småland	
	Loc. 1 Mixed pine forest	Loc. 2 Aspen forest	Loc. 3 Arable field	Loc. 4 Forest *Pinus silvestris*	Loc. 5 Forest *Picea abies*
Per cent of total nematode number	2.84	0.78	21.80	0.06	0.74
No. per ml soil	2.84	0.37	—	0.06	0.37
Occurrence in no. of sample units	69/146	16/62	27/34	1/11	3/12
Per cent	47.30	25.80	79.40	9.10	25.00

— figure not available.

In the mixed pine forest (locality 1) the mean monthly population density fluctuated between 0.43 and 6.06 with an annual mean value of 2.84 nematodes per *ml* (Fig. 1 a). The confidence limits are very wide indicating that the populations was quite aggregated. Fig. 1 a indicates that the population density increased during the late autumn. However this increase is not statistically significant.

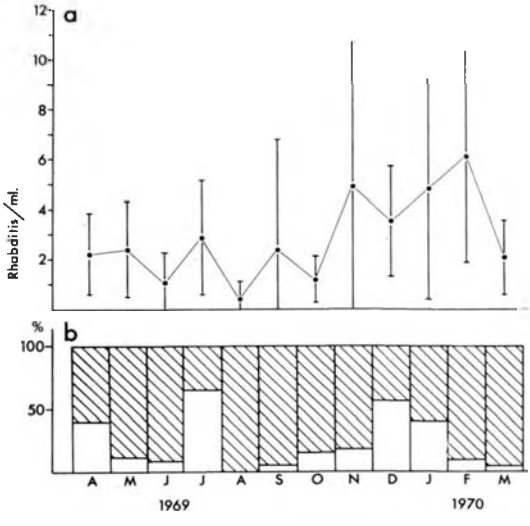

Fig. 1. (a) Fluctuation in the mean population of *Rhabditis* spp. (mean number per *ml* with 90 per cent confidence limits) between April 1969 and March 1970. (b) The percentage of active larvae (unfilled) and dauer larvae (striped) in the population.

Table 2 Composition of active stages and dauer larvae in populations of *Rhabditis* spp. from forest soil

Locality and period	Relative proportions (per cent) Adults	Active larvae	Dauer larvae	No. observed animals
Loc. 1. Mixed pine forest				
April 1969—March 1970	0	26.9	73.1	268
Loc. 2. Aspen forest in 1966				
May, June	7.5	37.5	55.0	40
July, August	1.8	49.1	49.1	55
September, October	6.1	60.6	33.3	66
The whole period	5.0	50.9	44.1	161

From studies on the relative frequencies of dauer larvae and active larvae (no adults were found) (Fig. 1 b) it is evident that the proportions of dauer larvae where high on most occasions. The frequency of these stages for the whole year can be seen in Tab. 2. Fig. 1 b shows that the proportion of active larvae in the samples increased during the later part of the autumn up to January. However high frequencies were also obtained in the April and July samples. These results and the increased population density indicate that the environmental conditions for growth improved during the autumn.

Also in the aspen forest (locality 2) the frequency of active stages was higher in the autumn samples (Table 2, Sept. and Oct.). In this locality the frequency of active stages was higher than in the mixed pine forest (locality 1) and some of the adults were carrying eggs during September and October.

4.2. Laboratory reared populations

Suspensions with nematodes extracted from the forest localities 1 and 2 were transferred to soil agar plates kept at 14 °C. In quite a lot of these plates dense Rhabditis populations occurred within rather short periods and these reached peak densities 2—3 weeks after inoculation. Thus these grew more rapidly than populations of any other nematode species and used up quite a great part of the food resources before the other bacterial feeding nematodes had begun to increase in number. Obviously these expanding Rhabditis populations had a retarding influence upon other nematode species. When the Rhabditis populations declined there was an increase in number of *Acrobeloides nanus*, *Plectus* sp. and *Cephalobus* sp. In those plates where no *Rhabditis* spp. occurred the other bacterial feeding nematodes expanded more rapidly and reached higher densities but the bacterial colonies were reduced much more slowly.

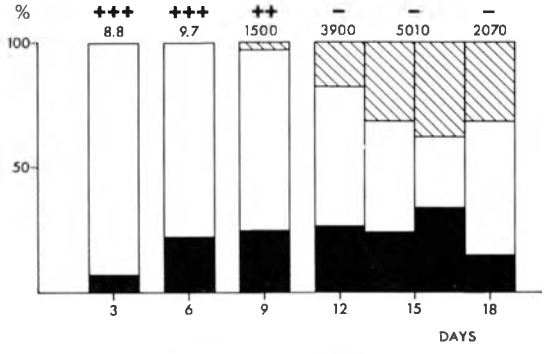

Fig. 2. The change in population structure in monoxenic agar culture with *Rhabditis terricola* started with five gravid adults (Adapted from Sohlenius 1969). The bars show number of active larvae (unfilled), dauer larvae (striped) and adults (solid). The figures over the bars indicate number of nematodes per *ml* substrate. +++ extensive, ++ moderate, — no visible bacterial growth.

Monoxenic cultures (*Escherichia coli* on Nigon's agar) were started with specimens of *Rhabditis* obtained from these soil agar cultures. The population development in these cultures resembled to a great extent that of *Rhabditis terricola* described in an earlier paper (Sohlenius 1969). A modified representation of the results from that study can be seen in Fig. 2. This figure shows that the first dauer larvae appeared before the visible bacterial growth had disappeared 9 days after inoculation and well before maximal population density (after 15 days). Later on the frequency of dauer larvae increased pronouncedly.

The structures of the Rhabditis populations from old cultures resembled those from forest soil because there were high proportions of dauer larvae. However the frequencies of adults were much higher in the laboratory populations.

4.3. Field experiment

The high proportion of dauer larvae as well as the low population densities in the Rhabditis populations from forest soils indicate that the conditions for growth and reproduction were bad. Probably this was mainly due to insufficient food supply. To see if this interpretation was correct the soil was locally enriched with organic material as was described in section 2.

This enrichment lead to an increase in total nematode number. From the figures obtained from the fungal bags (Table 3) it is evident that this increase was mainly due

Table 3 Changes in nematode numbers in connection with enrichment with fungal material. Figures show mean number per *ml* soil

	Untreated soil Oct.	Enriched soil. Days since start				Untreated soil Nov.
		8	16	23	35	
All nematodes	119.3	121.5	1610.0	1761.7	1150.7	156.9
Rhabditis spp.	1.2	8.4	1466.6	1629.2	1030.5	4.9
Acrobeloides nanus	10.8	18.0	18.8	57.6	39.9	16.9
All the other nematode species	107.3	95.1	124.6	74.9	80.3	135.1

to rapid increases in the number of *Rhabditis* spp. Also *Acrobeloides nanus* increased somewhat but the sum of the other nematode species decreased as a result of the enrichment. More detailed information of these changes of Rhabditis numbers is given in Tab. 4. The numbers in the soil around the bags increased relatively slowly in the beginning. Inside the bags however very high frequencies were found already on the first examinations 6—8 days after start. Later on the frequencies of *Rhabditis* spp. in the adjacent soil increased very pronouncedly especially around the bags with fungal material. The highest mean numbers were obtained after 23 days both for fungus and feces. At this time the number of *Rhabditis* spp. exceeded that of the untreated soil by about 300 to 500 times.

These pronounced increases in number were caused by growth and reproduction and to a minor extent by attraction from the surrounding soil. This conclusion is strongly supported when considering the frequencies of dauer larvae, active larvae and adults (Fig. 3). On the first examination 8 days after start no dauer larvae were found and the proportion of adults was high. This population structure resembles the structure of a population from a fresh agar culture, where the growth is not limited by the food supply (cf. Figs. 2 and 3). This therefore indicates that the conditions for growth and reproduction inside and around the fungal bags were good. The well filled intestines of the animals and the high number of uterine eggs in the adults also indicate this. Certainly the adults found 8 days after start originate from larvae that entered the fungal material chiefly as dauer larvae. As the population density in the undistrubed soil was quite low (Tab. 3 and Fig. 1) the high frequency of adults inside the fungal material indicates that the dauer larvae were attracted from rather long distances.

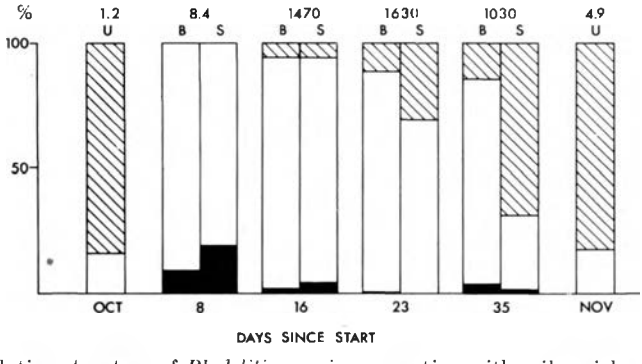

Fig. 3. The population structure of *Rhabditis* spp. in connection with soil enrichment with fungal material. The bars are marked as in Figs. 1 and 2. U shows the population from untreated soil, B the population from the interior of the fungal bags, S the population from soil adjacent to the bags. The figures over the bars show number of *Rhabditis* per *ml* soil in untreated soil (U) and in soil adjacent to the bags (S).

Dauer larvae were observed already on the second examination 16 days after inoculation (Fig. 3). The frequency of this stage increased somewhat inside the bags but was still low (about 15 per cent) on the last examination 35 days after start. In the adjacent soil the frequency of dauer larvae increased much more rapidly and was about 70 per cent on the last examination. The differences in frequencies of dauer larvae inside and outside the bags might indicate an outward migration of dauer larvae from the fungal material. Another explanation might be that the food supply was exhausted more rapidly in the surroundings than inside the bags.

5. Discussion and conclusions

The low densities and patchy distribution of the Rhabditis populations from forest soils correspond with results from earlier studies. Thus YEATES (1972) found that *Rhabditis* rarely exceeded one per cent of the nematode fauna in a Danish beech forest. Also VOLZ (1951) found rather low densities of *Rhabditis* in German oak and beech forest soil. In a Danish spruce forest with raw humus *Rabditis* just constituted about one per cent of the nematode fauna (NIELSEN 1949). In arable land however the situation seems to be quite another one. Thus SZCZYGIEL (1966) found *Rhabditis* spp. in 75—100 per cent of the sample units and this genus constituted 5—10 per cent of the nematode fauna in strawberry plantations. Also NIELSEN (1949) found high frequencies of *Rhabditis* spp. in rye and turnip fields where about 10 per cent of the fauna belonged to this genus. These results are in line with what was found in an arable field in the present study (Table 1 locality 3). The high frequencies of *Rhabditis* spp. in arable land may be due to manuring or are an effect of tilling.

Effects of enrichments have mostly been studied in laboratory cultures where soil and organic material have been mixed. DOUGHERTY and CALHOUN (1948) make a short review of the findings in this field and they also describe some enrichment experiments where different substances were tested. They found that a variety of substances can serve to enrich the soil populations of nematodes.

Enrichment in field was done by STÖCKLI (1952) who found that the resulting increase in nematode number was mainly due to *Rhabditis*. In a recent study DASH and CRAGG

Table 4 Effect of the application of bags with organic material upon number of *Rhabditis* spp. The figures show number per ml from soil adjacent to the bags

Days since start	6—8	14—16	23	35—36
Temp. °C	7.3	4.3	2.8	5.3
Untreated soil				
Mean	1.2	—	—	4.9
S. E.	0.54	—	—	3.20
Range	0—6.8	—	—	0—38.2
n	18			12
Feces				
Mean	2.5	22.5	1096	564
Range	1.4—3.6	2.9—42.0	22.2—2702	15.0—1073
n	2	2	3	3
Fungus				
Mean	8.4	1467	1629	1031
S. E.	7.56	701	615	225
Range	0—38.6	3.1—3118	86.1—3554	461—1582
n	5	5	5	5

— means that no samples were taken.

(1972) buried agar blocks with different fungi into the soil to study the effect of this on enchytraeids. In these studies also the number of nematodes increased. Agar blocks with bacteria (*Escherichia coli*) were used by Öhlin and Sohlenius (unpublished) in a study of winter activity in soil. In spite of the low temperatures (0 to +1 °C) there were pronounced population increases of *Rhabditis* spp. Also in the present study the field temperatures in connection with the enrichment experiment were rather low (Table 4). The results show that temperature may permit activity of *Rhabditis* spp. all round the year.

It seems probable that enrichment experiments can be further developed and can be used both to study food-relations and to study the influence of different environmental conditions upon nematode activity.

Earlier studies (Sohlenius 1968) show that some species belonging to the *maupasi* group can be cultured under very different conditions and are thus very tolerant towards different environmental conditions. However an important condition for active growth is obviously the presence of high numbers of suitable bacteria. Certainly there was a high bacterial activity in connection with both feces and fungi in the present enrichment experiment.

Obviously *Rhabditis* spp. are of slight importance under ordinary conditions in forest soil. However if there is a local flush in bacterial activity induced by deposition of easily decomposable organic material the importance of these nematodes improves greatly, which is indicated by their rapid increases in number and high peak densities. Their chief importance is probably as consumers and regulators of the bacterial populations. This is indicated by their ability to rapidly reduce bacterial populations in laboratory cultures (see Fig. 1 and Sohlenius 1969).

6. Acknowledgements

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7. Summary · Zusammenfassung

Rhabditis population densities from some different localities were estimated and found to be quite low in forest soils. The population structure expressed as the frequencies of dauer larvae active larvae and adults was determined. It was found that the populations from forest soil were most similar to the populations of old starving laboratory populations. The application of bags with organic material to soil gave rise to rapid increase in number and to changes in population structure. These changes in structure and density were similar to those of laboratory reared populations. Probably the ordinary soil conditions can support only a slight growth of these nematodes or one at all. Certainly they are dependent on local accumulations of organic material with high bacterial activity where local population increases can occur. When the food supplies in these loci are exhausted the surrounding soil is enriched with dauer larvae.

[Struktur und Dynamik von *Rhabditis* (Nematoda: Rhabditidae) aus Waldböden]

Die Besatzdichte von Rhabditis-Populationen in verschiedenen Biotopen wurde geschätzt und in Waldböden als ziemlich gering befunden. Die Populationsstruktur, ausgedrückt als Frequenz der Dauerlarven, aktiven Larven und Adulten, wurde bestimmt. Es wurde gefunden, daß die Populationen aus Waldböden denen von hungernden alten Laboratoriums-Populationen am meisten ähnlich waren. Die Einbringung von Gazebeuteln mit organischem Material ergab einen starken Anstieg der Besatzdichte im Boden und Veränderungen der Besatzstruktur. Diese Änderungen der Struktur und Besatzdichte waren denen von im Laboratorium gezogenen Populationen ähnlich. Wahrscheinlich können die gewöhnlichen Bodenbedingungen die Entwicklung dieser Nematoden nicht oder nur wenig fördern. Sicherlich sind die Nematoden aus örtlichen Akkumulationen von organischem Material mit hoher bakterieller Aktivitität, in denen lokale Vermehrungen der Populationen möglich sind, abhängig. Wenn die Nahrungsvorräte in diesen Lokalitäten aufgebraucht sind, ist der umgebende Boden mit Dauerlarven angereichert.

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